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(71) Applicant *(for all designated States except US)*: UNIVERSITY OF UTAH RESEARCH FOUNDATION [US/US]; 210 Park Building, Salt Lake City, UT 84112 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LIU, Feng [CN/US]; Apt. 2, 247 South 1100 East, Salt Lake City, UT 84102 (US). BAUDYS, Miroslav [CZ/US]; 905 University Village, Salt Lake City, UT 84108 (US). KIM, Sung, Wan [US/US]; 1711 Devonshire, Salt Lake City, UT 84108 (US).

(74) Agents: HOWARTH, Alan, J. et al.; Clayton, Howarth & Cannon, P.C., P.O. Box 1909, Sandy, UT 84091–1909 (US).

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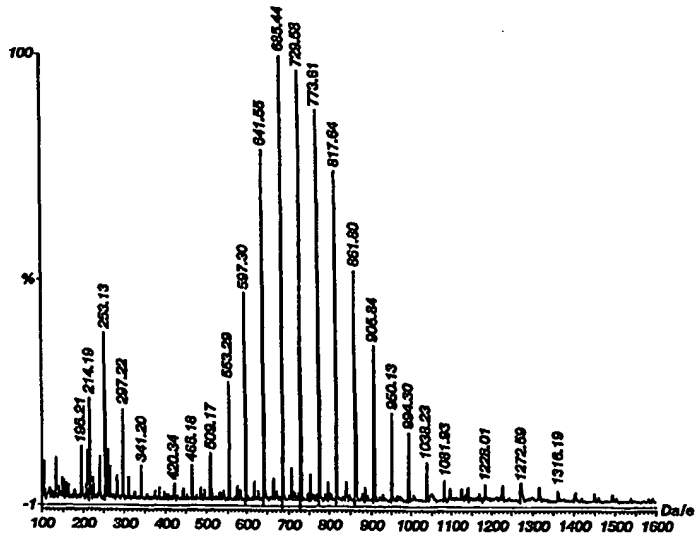

(57) Abstract

A method of making low molecular weight polyethylene glycol carboxylic acids comprises reacting lower molecular weight polyethylene glycols with monohalogen–substituted aliphatic acid esters in base, and then saponifying the resulting intermediates. The polyethylene glycol carboxylic acids are then extracted with a toluene–containing organic phase for removing unreacted compounds and byproducts. The extracted polyethylene glycol carboxylic acids are then acidified and purified by dialysis, gel filtration, or extraction and precipitation.

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METHOD FOR PREPARATION OF LOW MOLECULAR WEIGHT POLYETHYLENE GLYCOL-CARBOXYLIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/098,802, filed September 2, 1998.

BACKGROUND OF THE INVENTION

This invention relates to polyethylene glycol derivatives. More particularly, the invention relates to a mild and very efficient method of preparation of high purity, low molecular weight polyethylene glycol derivatives having one or more terminal carboxylic acid groups. These polyethylene glycol derivatives are useful for preparation of conjugates of low molecular weight polyethylene glycol with peptides or proteins.

A growing number of pharmaceutically relevant peptide and protein drugs are becoming available for use in medical therapies. Most of them, however, exhibit some specific properties that make their use in disease treatment questionable and problematic. To improve peptide and protein drug parameters, especially pharmacokinetic, pharmacodynamic, and immunogenic properties, hydrophilic macromolecular compounds have been coupled to peptide and protein moieties. Among these hydrophilic macromolecular compounds, polyethylene glycol (PEG) and its derivatives are the most frequently used for such purposes. It has been demonstrated that proteins with PEG attached (i.e., PEG-proteins, made by reaction of the protein with an active PEG) remain active and have a greatly diminished or negligible immune response. The result is that these PEG-proteins have greatly increased serum lifetimes. In addition, PEG attachment makes proteins much larger and thus reduces their rate of clearance through the kidney. PEG also is nontoxic and has been approved by the U.S. Food and Drug Administration (FDA) for topical and internal use in humans. PEG is soluble in water and many organic solvents, and it forms aqueous two-phase systems when paired with certain other polymers (such as dextran). It is insoluble in ethyl ether and hydrocarbons such as hexane. The water solubility, lack of toxicity, high flexibility and well-defined chemistry of PEG having a carboxylic acid functional group makes it ideally suited for many cross linking or tethering applications.

Among PEG derivatives used for protein modification, the activated intermediates of alkoxyPEG-carboxylic acids are most frequently applied. U.S. Patent No. 4,179,337. This requires that a carboxylic group be substituted for a hydroxyl group at an end of the alkoxyPEG chain. One of two general synthetic procedures, an oxidative method or a substitution method, is usually used. The oxidative method suffers from side reactions that lead to PEG chain fragmentation, and therefore it is not frequently used. G. Johansson et al., Proc. Int. Solvent Extract. Conf., Lyon, 927-942 (1974). The milder and thus preferred substitution method, G. Royer et al., 101 J. Amer. Chem. Soc. 3394-3396 (1979), uses esters of halogen monosubstituted aliphatic acids, such as ethyl bromoacetate, in the presence of base. The resulting esters of PEG-carboxylic acid are saponified and the product, PEG-carboxylic acid, is purified by repetitive precipitation in the excess of a nonsolvent, such as ether or a mixture of ether/ethanol, which enables the quantitative removal of reaction byproducts. Nevertheless, unreacted alkoxyPEG cannot be separated and will contaminate the product.

Usually, commercially available PEG derivatives with high molecular weight (3,500 or higher) are used. This often leads, however, to diminished bioactivity of a peptide or protein drug because of the steric hindrance of the long PEG chains. The long chain PEG portions of the molecule create steric hindrances that interfere with receptor or substrate binding. It is becoming more and more evident that low molecular weight PEG derivatives (M.Wt. range 200- 2,000) are better suited for tailoring peptide and protein drug properties. F. Liu et al., 38 Polymer Preprints 595-596 (1997). The use of low molecular weight PEG derivatives has been prevented in the past, mainly due to the difficulties related to their synthesis and, more importantly, to their purification, especially removal of reaction byproducts.

In view of the foregoing, it will be appreciated that providing an efficient method for the synthesis of low molecular weight polyethylene glycol-carboxylic acids, wherein byproducts and unreacted PEG can easily be removed, would be a significant advancement in the art.

BRIEF SUMMARY OF THE INVENTION

It is an object of the present invention to provide a process for synthesis of low

molecular weight PEG-dicarboxylic acids and alkoxyPEG-carboxylic acids and their homologues, wherein the reaction conditions are mild, few byproducts are produced, the reaction is efficient, and unreacted PEG can easily be removed and recycled.

These and other objects can be achieved by providing a method for making and purifying a low molecular weight polyethylene glycol carboxylic acid comprising:

(a) reacting, in the presence of aqueous base, a polyethylene glycol having the formula:

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\underset{R_2}{|}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\underset{R_2}{|}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 is lower alkyl, R_2 is H or lower alkyl, m is an integer of about 2 to 12, and n is an integer of about 3 to 120, with a monohalogen substituted aliphatic ester having the formula:

$$XH_2C\text{-}(CH_2)_p\text{-}COOR_3$$

wherein X is Cl, Br, or I; p is an integer of 0 to about 8; and R_3 is lower alkyl, to result in an ester of a low molecular weight polyethylene glycol carboxylic acid;

(b) saponifying the ester of a low molecular weight polyethylene glycol carboxylic acid to result in the low molecular weight polyethylene glycol carboxylic acid, contained in an aqueous phase, having a formula selected from the group consisting of:

$$HOOC\text{-}(CH_2)_{p+1}\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\underset{R_2}{|}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$

and

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\underset{R_2}{|}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH.$$

(c) extracting the low molecular weight polyethylene glycol carboxylic acid-containing aqueous phase with a toluene-containing, water immiscible organic phase for removing unreacted compounds and byproducts from the aqueous phase;

(d) separating the extracted aqueous phase from the organic phase and acidifying the extracted aqueous phase;

(e) desalting the separated aqueous phase; and

(f) removing water from the aqueous phase to result in a purified low molecular weight polyethylene glycol carboxylic acid.

The toluene-containing, water immiscible organic phase preferably comprises chloroform/toluene in a ratio of about 1/99 to 99/1 by volume, and more preferably in a ratio of about 95/5 by volume. The desalting is preferably carried out by dialysis or gel filtration, and the removing of water from the aqueous phase is preferably carried out under reduced pressure.

In another aspect of the invention, a method for making and purifying a low molecular weight polyethylene glycol carboxylic acid comprises:

(a) reacting, in the presence of aqueous base, a polyethylene glycol having the formula:

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 is lower alkyl, R_2 is H or lower alkyl, m is an integer of about 2 to 12, and n is an integer of about 3 to 120, with a monohalogen substituted aliphatic ester having the formula:

$$XH_2C\text{-}(CH_2)_p\text{-}COOR_3$$

wherein X is Cl, Br, or I; p is an integer of 0 to about 8; and R_3 is lower alkyl, to result in an ester of a low molecular weight polyethylene glycol carboxylic acid;

(b) saponifying the ester of a low molecular weight polyethylene glycol carboxylic acid to result in the low molecular weight polyethylene glycol carboxylic acid, contained in an aqueous phase, having a formula selected from the group consisting of:

$$HOOC\text{-}(CH_2)_{p+1}\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$

and

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\underset{\displaystyle R_2}{|}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH.$$

(c) extracting the low molecular weight polyethylene glycol carboxylic acid-containing aqueous phase with a first toluene-containing, water immiscible organic phase for removing unreacted compounds and byproducts from the aqueous phase;

(d) separating the extracted aqueous phase from the first organic phase and acidifying the extracted aqueous phase;

(e) next extracting the separated aqueous phase with a second toluene-containing, water immiscible organic phase such that the low molecular weight polyethylene glycol carboxylic acid is extracted into the second organic phase, and removing the aqueous phase;

(f) evaporating the second organic phase containing the polyethylene glycol carboxylic acids;

(g) redissolving the polyethylene glycol carboxylic acids in an organic solvent;

(h) precipitating the polyethylene glycol carboxylic acids from the organic solvent by extraction with a nonpolar precipitation solvent; and

(i) collecting and drying the precipitated polyethylene glycol carboxylic acids, thereby obtaining a purified low molecular weight polyethylene glycol carboxylic acid.

The first and second toluene-containing, water immiscible organic phases preferably comprise chloroform/toluene in a ratio of about 1/99 to 99/1 by volume, and more preferably about 95/5 by volume. The organic solvent is preferably a member selected from the group consisting of acetone, ethanol, chloroform, methylene chloride, and mixtures thereof. The nonpolar precipitation solvent is preferably diethyl ether.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 shows Fourier transform infrared (FTIR) spectra of CH_3O-PEG-OH (mPEG; lower spectrum) and its carboxy-methylated derivative, CH_3O-PEG-O-CH_2-COOH (upper spectrum, wherein the new band at 1754 cm^{-1} originated from the introduced COOH group.

FIG. 2 shows a ^{1}H NMR spectrum of mPEG-OCH_2-COOH.

FIG. 3 shows a MALDI MS spectrum of mPEG-OCH_2-COOH.

DETAILED DESCRIPTION

Before the present methods for synthesis of low molecular weight polyethylene glycol carboxylic acids are disclosed and described, it is to be understood that this invention is not limited to the particular configurations, process steps, and materials disclosed herein as such configurations, process steps, and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

The publications and other reference materials referred to herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polyethylene glycol" includes references to two or more of such polyethylene glycols, and reference to "an aliphatic ester" includes reference to two or more of such aliphatic esters.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out herein.

As used herein, "comprising," "including," "containing," "characterized by," and grammatical equivalents thereof are inclusive or open-ended terms that do not exclude additional, unrecited elements or method steps. "Comprising" is to be interpreted as including the more restrictive terms "consisting of" and "consisting essentially of."

As used herein, "consisting of" and grammatical equivalents thereof exclude any element, step, or ingredient not specified in the claim.

As used herein, "consisting essentially of" and grammatical equivalents thereof limit the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic or characteristics of the claimed invention.

As used herein, "peptide" means peptides of any length and includes proteins. The terms "polypeptide" and "oligopeptide" are used herein without any particular intended size limitation, unless a particular size is otherwise stated. Typical of peptides that can be utilized are those selected from group consisting of oxytocin, vasopressin, adrenocorticotrophic

hormone, epidermal growth factor, prolactin, luliberin or luteinizing hormone releasing hormone, growth hormone, growth hormone releasing factor, insulin, somatostatin, glucagon, interferon, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, renin, bradykinin, bacitracins, polymixins, colistins, tyrocidin, gramicidins, and synthetic analogues, modifications and pharmacologically active fragments thereof, monoclonal antibodies and soluble vaccines. The only limitation to the peptide or protein that may be utilized is one of functionality.

As used herein, "low molecular weight" refers to PEG and PEG derivatives having a molecular weight in the range of about 200 to 2000. As used herein, "high molecular weight" refers to PEG and PEG derivatives having a molecular weight of greater than about 3,500.

As used herein, "PEG" means polyethylene glycols and derivatives thereof having at least one terminal hydroxyl group. Included in the definition of PEG are star PEGs and branched PEGs, such as are commercially available from Shearwater Polymers, Inc. (Huntsville, Alabama). Star PEGs are multi-armed PEGs that can be made by polymerization of ethylene oxide from a cross-linked divinyl benzene core. Y. Gnanou et al., 189 Makromol. Chem. 2885 (1988); D. Rein et al., 44 Acta Polymer. 225 (1993). Branched PEGs can be prepared by ethoxylation of various polyols (derived from glycerol condensation) having 3 to 10 arms (pPEGs). Especially preferred PEGs, however, are low molecular weight PEGs having the formula:

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(CH(R_2)\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(CH(R_2)\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 represents lower alkyl, R_2 represents H or lower alkyl, n is an integer of about 3 to 120 and m is an integer of about 2 to 12.

As used herein, "lower alkyl" means a straight or branched alkyl chain of 6 or fewer carbons.

As used herein, "NMR" means nuclear magnetic resonance spectroscopy.

As used herein, "FTIR" means Fourier transform infrared spectroscopy.

As used herein, "MALDI MS" means matrix assisted laser desorption/ionization mass spectrometry.

In an illustrative embodiment of the invention, a method for making low molecular weight PEG-dicarboxylic acids or alkoxyPEG-carboxylic acids and their homologues starting from low molecular weight polyethylene glycol derivatives having the general formula

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 represents lower alkyl, R_2 represents lower alkyl or H, n is an integer of about 3 to 120 and m is an integer of about 2 to 12, comprises reacting them in the presence of aqueous base with monohalogen substituted aliphatic acid esters having the formula

$$XH_2C\text{-}(CH_2)_p\text{-}COOR_3$$

wherein X is a halogen atom, preferably Cl, Br, or I, p is an integer from 0 to about 8, and R_3 is lower alkyl, using any of several well known substitution reactions giving rise, after ester saponification, to the product. The product produced by the above reaction is either low molecular weight PEG-dicarboxylic acid or alkoxyPEG-carboxylic acid and their derivatives having the formula

$$HOOC\text{-}(CH_2)_{p+1}\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$

wherein R_1, R_2, n, m, and p are as specified above.

The reaction mixture, dissolved in aqueous base, is extracted into an organic phase

composed of a chloroform/toluene mixture, causing removal of unreacted PEG derivatives and other reaction byproducts, which are often colored. The proportions of chloroform and toluene are limited only by functionality, preferably in the range of about 1/99 to 99/1 by volume, more preferably about 95/5 by volume. The toluene in the predominantly chloroform-based organic phase appears to be required for fast and easy separation of the two immiscible phases. The product contained in the aqueous phase is acidified and further purified either by dialysis or gel filtration to remove small molecular weight compounds, such as halogen-substituted aliphatic acids. The final product can be obtained directly by lyophilization of the resultant dialysate or filtrate.

Alternatively, the product in the water phase is first acidified and then extracted into chloroform/toluene solution, preferably 95/5 by volume. Following the extraction, the organic phase is removed by evaporation. Finally, the product is dissolved in a minimum volume of a solvent, such as acetone, ethanol, chloroform, or methylene chloride; precipitated by the addition of a nonpolar solvent, such as ether; and kept at subzero temperature, preferably -20°C or lower for between several minutes to several hours, sufficient to cause the complete precipitation of the product. Precipitated product is collected, preferably by centrifugation at -20°C or lower. If necessary because of residual product remaining in the mother liquor, the precipitation procedure can be repeated. The final product is dried under reduced pressure to remove solvents.

The PEG carboxylic acids prepared according to the present method can be activated by conversion to acid chloride or tresylate derivatives according to methods well known in the art. These activated PEG carboxylic acids can then be reacted with peptides according to well known methods to result in PEG-peptide conjugates. A preferred reaction is the formation of an amide bond between the activated carboxylic acid moiety of the PEG carboxylic acid and an α-amino or ε-amino group of the peptide.

Following is an example that will enable one of ordinary skill in the art to practice the invention. Specifically, the following example demonstrates how the lower molecular weight PEG-carboxylic acids of the present invention may be synthesized.

Example

First, 10.0 grams (18.2 mmol) of methoxypolyethylene glycol (mPEG) (molecular

weight 550) was dissolved in 80 ml of *tert*-butyl alcohol. Then, 3.06 grams (27.3 mmol) of potassium *tert*-butoxide was added to the mPEG solution. The resulting mixture was purged with nitrogen for 10 minutes and stirred at 40 to 50°C for 20 minutes. Next, 4.56 g (27.3 mmol) of ethyl bromoacetate dissolved in 20 ml of *tert*-butyl alcohol was added to the mixture over a period of 5 minutes at 40°C to 50°C. The residue was dissolved in 100 ml of distilled water containing 1.2 gram of NaOH, and the aqueous solution was then stirred at room temperature for one hour. The unreacted fraction of mPEG was extracted in 50 ml of an immiscible organic phase composed of chloroform and toluene (95:5, v/v). The extraction procedure was repeated three times. The water phase was then acidified with 6 N HCl and the pH brought to 2.6. The product, methoxy carboxymethyl polyethylene glycol, was extracted four times from the acidic water phase with 50 ml of a mixture composed of chloroform and toluene (95:5, v/v). Next, the pooled extraction organic phase was washed twice with 25 ml of distilled water and dried over sodium sulfate. The organic solvent was evaporated under vacuum at a temperature of 70°C to 80°C. The residue was dissolved in a minimal volume of ethanol (5 to 10 ml) and this solution was poured into 50 ml of dry ether kept at a temperature of -20°C. The final precipitated product was collected by centrifugation at -20°C and dried under high vacuum. The overall yield was from 40 to 50%.

Gel permeation chromatography data demonstrated that no cleavage of polyethylene glycol chains occurred. FTIR spectra of the starting mPEG and the final mPEG carboxylic acid are shown in FIG. 1. The new band at 1754 cm^{-1} for the product clearly indicates the presence of a newly introduced carboxylic acid group. FIG. 2 shows a ^1H NMR spectrum for mPEG carboxylic acid, wherein the signal at 4.1 ppm originates from the methylene group of the $-CH_2-COOH$ group. Further, the area ratio for this group and the signal corresponding to protons of the methoxy group at 3.2 ppm is 0.627, a value close to theoretical 0.667 (2/3). Finally, a MALDI MS spectrum (FIG. 3) of the product demonstrated its purity as well as polydispersity, since the starting mPEG is polydisperse. Thus, molecular weights of individual molecular ions (CH_3O-PEG-CH_2-$COOHNa^+$) differ by 44 Da, corresponding to the molecular weight of the repeated $-CH_2-CH_2-O-$ unit. Volumetric titration analysis data showed that the carboxylic acid content was over 98 mol% of the product.

CLAIMS

What is claimed is:

1. A method for making and purifying a low molecular weight polyethylene glycol carboxylic acid comprising:

(a) reacting, in the presence of aqueous base, a polyethylene glycol having the formula:

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\substack{|\\ R_2}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\substack{|\\ R_2}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 is lower alkyl, R_2 is H or lower alkyl, m is an integer of about 2 to 12, and n is an integer of about 3 to 120, with a monohalogen substituted aliphatic ester having the formula:

$$XH_2C\text{-}(CH_2)_p\text{-}COOR_3$$

wherein X is Cl, Br, or I; p is an integer of 0 to about 8; and R_3 is lower alkyl, to result in an ester of a low molecular weight polyethylene glycol carboxylic acid;

(b) saponifying said ester of a low molecular weight polyethylene glycol carboxylic acid to result in said low molecular weight polyethylene glycol carboxylic acid, contained in an aqueous phase, having a formula selected from the group consisting of:

$$HOOC\text{-}(CH_2)_{p+1}\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\substack{|\\ R_2}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$

and

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\substack{|\\ R_2}}{CH}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH.$$

(c) extracting the low molecular weight polyethylene glycol carboxylic acid-containing aqueous phase with a toluene-containing, water immiscible organic phase for

removing unreacted compounds and byproducts from the aqueous phase;

(d) separating said extracted aqueous phase from said organic phase and acidifying said extracted aqueous phase;

(e) desalting said separated aqueous phase; and

(f) removing water from said aqueous phase to result in a purified low molecular weight polyethylene glycol carboxylic acid.

2. The method of claim 1 wherein said toluene-containing, water immiscible organic phase comprises chloroform.

3. The method of claim 2 wherein said toluene-containing, water immiscible organic phase comprises chloroform/toluene in a ratio of about 1/99 to 99/1 by volume.

4. The method of claim 3 wherein said toluene-containing, water immiscible organic phase comprises chloroform/toluene in a ratio of about 95/5 by volume.

5. The method of claim 1 wherein said desalting is carried out by dialysis or gel filtration.

6. The method of claim 1 wherein said removing water from said aqueous phase is carried out under reduced pressure.

7. A method for making and purifying a low molecular weight polyethylene glycol carboxylic acid comprising:

(a) reacting, in the presence of aqueous base, a polyethylene glycol having the formula:

$$HO\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

or

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(\underset{\displaystyle R_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}OH$$

wherein R_1 is lower alkyl, R_2 is H or lower alkyl, m is an integer of about 2 to 12, and n is an integer of about 3 to 120, with a monohalogen substituted aliphatic ester having the formula:

$$XH_2C\text{-}(CH_2)_p\text{-}COOR_3$$

wherein X is Cl, Br, or I; p is an integer of 0 to about 8; and R_3 is lower alkyl, to result in an ester of a low molecular weight polyethylene glycol carboxylic acid;

(b) saponifying said ester of a low molecular weight polyethylene glycol carboxylic acid to result in said low molecular weight polyethylene glycol carboxylic acid, contained in an aqueous phase, having a formula selected from the group consisting of:

$$HOOC\text{-}(CH_2)_{p+1}\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}(CH\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad R_2$$

and

$$R_1O\text{-}(CH_2)_m\text{-}O\text{-}(CH\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_m\text{-}O\text{-}(CH_2)_{p+1}\text{-}COOH.$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad R_2$$

(c) extracting the low molecular weight polyethylene glycol carboxylic acid-containing aqueous phase with a first toluene-containing, water immiscible organic phase for removing unreacted compounds and byproducts from the aqueous phase;

(d) separating said extracted aqueous phase from said first organic phase and acidifying said extracted aqueous phase;

(e) next extracting the separated aqueous phase with a second toluene-containing, water immiscible organic phase such that the low molecular weight polyethylene glycol carboxylic acid is extracted into the second organic phase, and removing the aqueous phase;

(f) evaporating the second organic phase containing the polyethylene glycol carboxylic acids;

(g) redissolving said polyethylene glycol carboxylic acids in an organic solvent;

(h) precipitating said polyethylene glycol carboxylic acids from said organic solvent by extraction with a nonpolar precipitation solvent; and

(i) collecting and drying said precipitated polyethylene glycol carboxylic acids, thereby obtaining a purified low molecular weight polyethylene glycol carboxylic acid.

8. The method of claim 7 wherein said first toluene-containing, water immiscible organic phase comprises chloroform.

9. The method of claim 8 wherein said first toluene-containing, water immiscible organic phase comprises chloroform/toluene in a ratio of about 1/99 to 99/1 by volume.

10. The method of claim 9 wherein said first toluene-containing, water immiscible organic phase comprises chloroform/toluene in ratio of about 95/5 by volume.

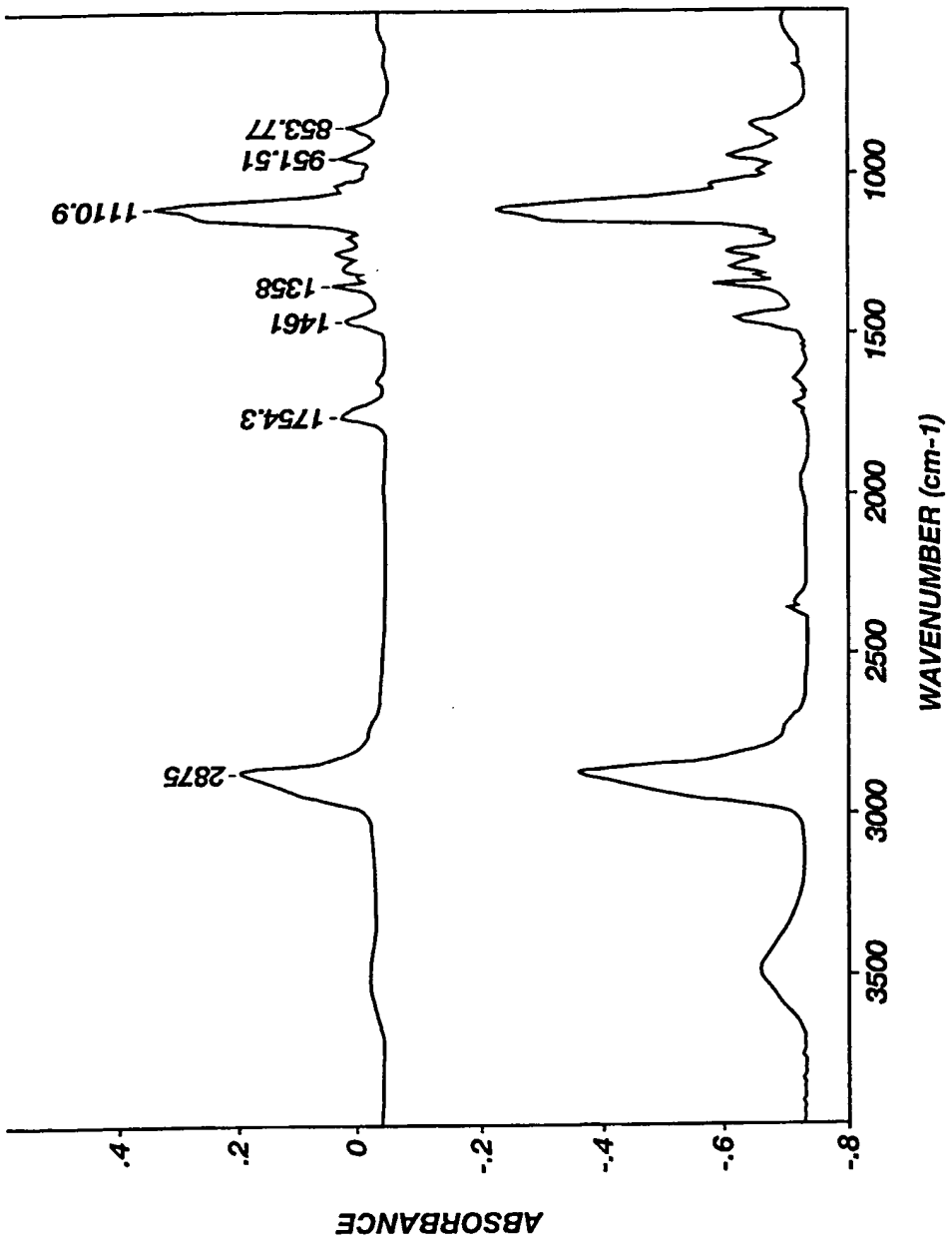
11. The method of claim 7 wherein the second toluene-containing, water immiscible organic phase comprises chloroform.

12. The method of claim 11 wherein said second toluene-containing, water immiscible organic phase comprises chloroform/toluene in a ratio of about 1/99 to 99/1 by volume.

13. The method of claim 12 wherein said second toluene-containing, water immiscible organic phase comprises chloroform/toluene in a ratio of about 95/5 by volume.

14. The method of claim 7 wherein said organic solvent is a member selected from the group consisting of acetone, ethanol, chloroform, methylene chloride, and mixtures thereof.

15. The method of claim 7 wherein said nonpolar precipitation solvent is diethyl ether.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.2
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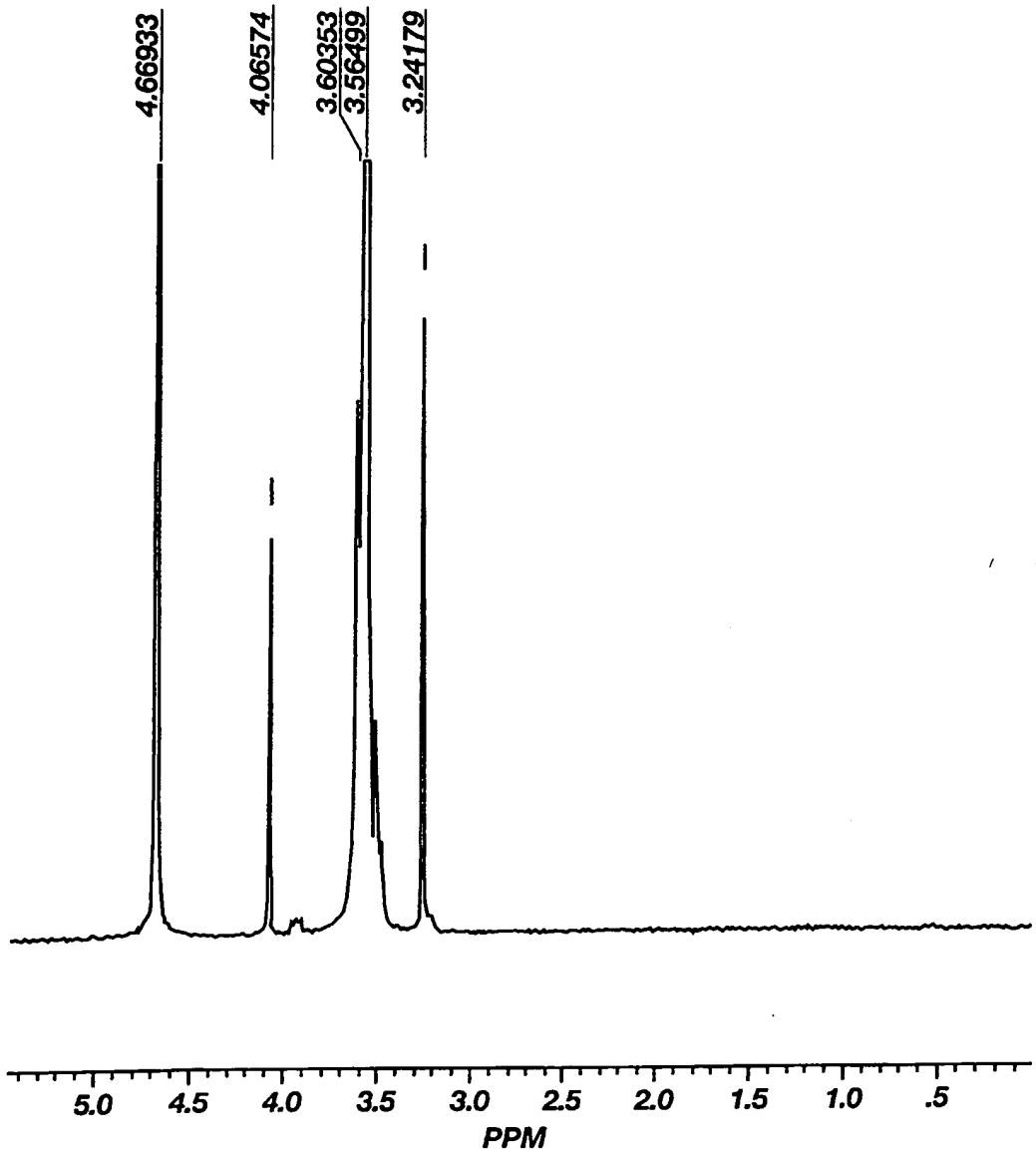
-.4
-.6
-.8
3500
3000
2500
2000
1500
1000
WAVENUMBER (cm-1)

Fig. 1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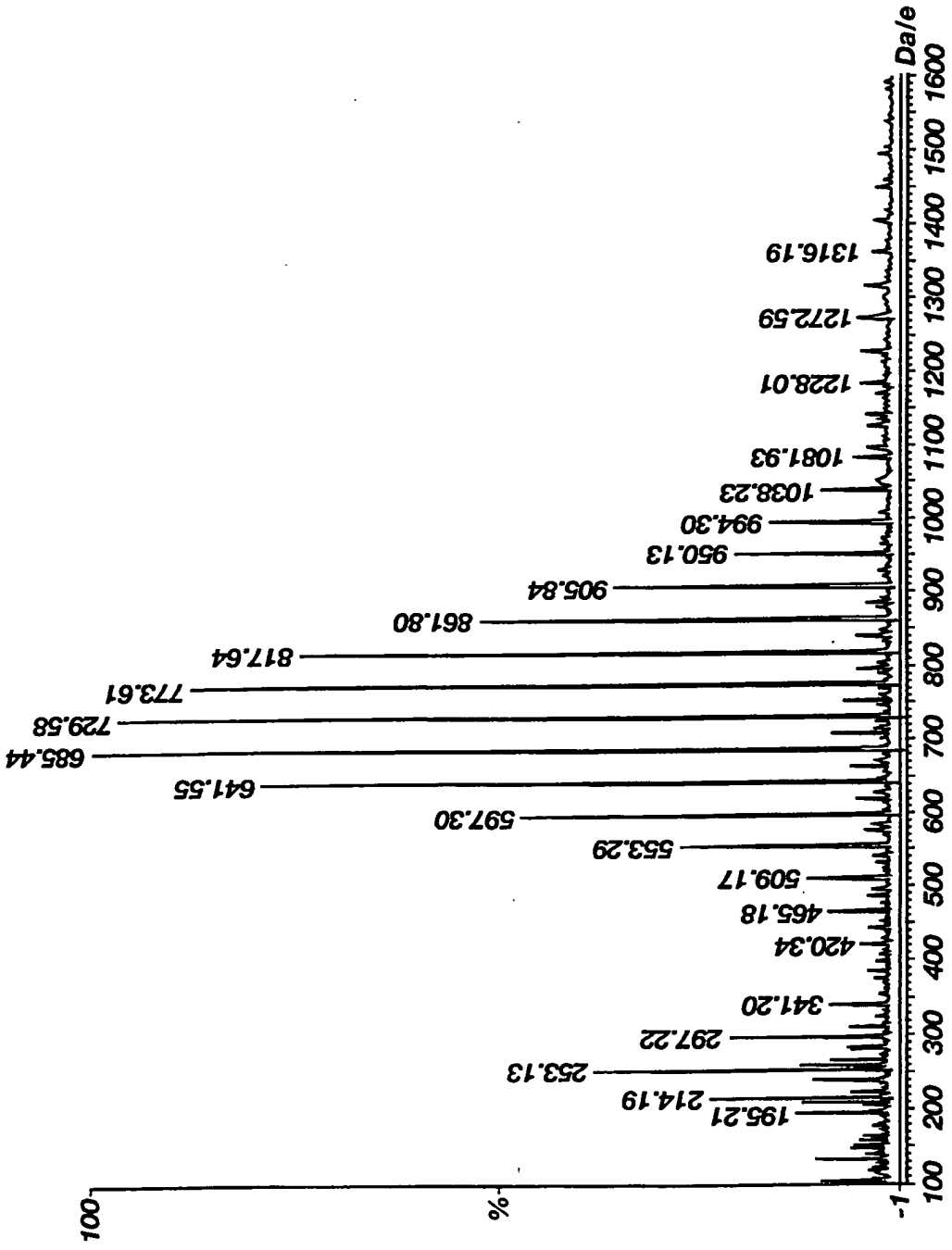
1.0
.5
PPM

Fig. 2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465.18
509.17
553.29
597.30
641.55
685.44
729.58
773.61
817.64
861.80
905.84
950.13
994.30
1038.23
1081.93
1228.01
1272.59
1316.19

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20135

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07C 59/10, 59/285

US CL : 562/587

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 562/587

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST:search terms: polyethylene glycol, base (e.g. NaOH), chloracetic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2,623,900 A (HOFER et al.) 30 December 1952, col. 3, lines 55-75.	1
Y	US 3,992,443 A (SPRINGMANN) 16 November 1976, col. 7, lines 1-27; col. 6, lines 17-19; col. 9, lines 62-63.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 JANUARY 2000	Date of mailing of the international search report 07FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T. Victor Oh Telephone No. (703) 308-1235

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